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Antibacterial and Toxicity Evaluation of Eastern Medicine Formulation Eczegone for the Management of Eczema

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Abstract

The present study was conducted to evaluate the antibacterial activity, *in vitro* and *in vivo* cytotoxicity, cell viability and safety of Eastern Medicine coded medicinal formulation Eczegone comprising extracts of *Azadirachta indica* (Azin), *Fumaria indica* (Fuin), *Sphaeranthus indicus* (Spin) and *Lawsonia inermis* (Lain). This work also evaluated antibacterial activity of Eczegone formulation having above mentioned plants ethanolic extracts against different bacteria's by disk diffusion method. *In vitro* toxicity of Eczegone formulation was investigated by using human skin keratinocytes HaCaT cell line, crystal violet stained cells, and methyl tetrazolium cytotoxicity (MTT) assay. *In vivo* acute oral and dermal cytotoxicity was determined by using Swiss albino mice and albino rabbits, respectively. The Eczegone formulation showed antibacterial activity against 3 gram negative bacteria including *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris* and a gram positive *Staphylococcus aureus*. We didn't observe any toxic effect of Eczegone formulation on the skin keratinocytes. Furthermore, the Ezcegone formulation was non-irritant according to draize score (OECD TG404, 2002). After rigorous safety evaluation by *in vitro* and *in vivo* acute oral and dermal toxicity analysis, we concluded that Eczegone formualtion possessses antibacterial effects and is safe, non-toxic, non-irritant, and the drug would be subjected for further biochemical and clinical studies.

Keywords

eczegone, antibacterial activity, MTT assay, acute oral and dermal toxicity, cell viability

Introduction

Eczema or atopic dermatitis has become one of the most common skin problems globally.[1] The prolonged involvement of the patients in atopic dermatitis disturbs the quality of life significantly, creates economic and social setbacks in families.[2,3] According to the WHO Global Burden of Diseases, 230 million people have atopic dermatitis with 3.5% global annual period prevalence.[4] Many countries have lifetime prevalence of even more than 15%,[5] 10-25% in children, and 2-8% in adults. Among them, 25% of the victims have moderate to severe illness.[6,7] Annual cost for the treatment of the atopic dermatitis in United States is approximately $5.297.[8]

There are no specific treatment options available for the management of eczema except topical corticosteroids and calcineurin inhibitors that can provide only symptomatic relief. However, such treatment options have been associated with different adverse events, and drug tolerance.[9] Topical steroids have been usually administrated to treat moderate to severe diseases which unfortunately cause a series of side effects during their

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long term use at high doses.[10] Corticosteroids act as anti-inflammatory agents and work by helper T-cells (Th2) dominant immune response.[11,12] Nowadays herbal formulations in huge quantities are being used in developed countries as compared to synthetic medicine for the management of different types of skin ailments such as wound healing, scabies, swelling and boils etc.[13-20] In Pakistan, different herbal medicines are available and used for the management of various skin diseases[19] and chronic diseases including hepatitis,[21-23] Alzheimer disease,[22] kidney failure,[24] hypercholesterolemia,[25] muscular pain,[26] hyperuricemia,[27] obesity[28,29] and insulin sensitivity.[30] Hence a potent drug to overcome the skin conditions is a matter of indeed. Previous reports showed that extract Azadirachta indica (*Azin*), *Sphaeranthus indicus* (*Spin*) and *Fumaria indica* (*Fuin*) possesses therapeutic activities in disease management including through modulation of various activities including antioxidant, antibacterial, anti-inflammatory, anti-cancerous, wound healing, hepatoprotective, antinephrotoxicity, and immunomodulatory activities etc.[31-34] Previous reports also showed that extracts of *Lawsonia inermis* (*Lain*) possesses various biological activities including improvement in the symptoms of contact dermatitis.[35,36] The present study was aimed to test an Eastern Medicine coded plant based medicinal formulation Eczegone ointment comprising of *Azin, Fuin, Spin* and *Lain* for its toxicity evaluation and clinical effects for atopic dermatitis. The toxicity studies were performed by using Human Skin Keratinocytes HaCaT cell line by treating them with different concentrations of Eczegone. The morphological changes for any toxicity were observed after treating with HaCaT cells and crystal violet stained cells used to find out the safety of Eczegone on skin cells and their cell viability by using Methyl tetrazolium cytotoxicity (MTT) assay. Furthermore, the samples of Eastern Medicine coded formulation Eczegone in the form of both tablet and ointment were tested using acute oral and dermal toxicity tests on Swiss albino mice and albino rabbits with different drug concentrations. In addition to safety and toxicity evaluation of Eczegone, we also aimed to asses antibacterial activity of Eczegone whether it possess antibacterial activity or not. To check any potential of Eczegone against the bacterial growth of both gram-positive and gram-negative nature, antibacterial activity was conducted in the current study.

Methodology

Collection of Plant Material

The crude drugs were purchased from the local market and identified by Pakistan Museum of Natural History as *Azin* (voucher number PMNH-ISD-05), *Lain* (voucher number PMNH-ISD-06), *Fain* (voucher number PMNH-ISD-07) and *Spin* (voucher number PMNH-ISD-08).

Preparation of Plant Extract

We used leaves of Azin or barg-e-neem and Lain or barg-e-hena, whole plant of Fuin or shahtra, and flowers of Spin or gul mundi for extraction. The herbal extract formulation was prepared at Faculty of Eastern Medicine, Hamdard University, Karachi. Herbs were washed, dried in air, and processed by continuous extractions. *Azin, Fuin, Spin* and *Lain* were analyzed by the basic quality control according to Herbal Pharmacopoeia. These herbs were mixed in ratio of 1:1:1:1, respectively and extracted in 95% ethanol for 7 days at room temperature. Then, this extract solution was subjected into rotary evaporator, and it was freeze-dried to remove the solvent. Finally, the extract of the formulation was sterilized at 20°C.

Chemicals

Ethanol, dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthia-zol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), fetal bovine serum (FBS) and Mueller-Hinton agar were purchased from Sigma, St. Louis, Mo., USA. Dalbeccos modified eagles medium (DMEM)-low glucose was purchased from Wako, Osaka, Japan. Ciprofloxacin was purchased from the Sami Pharmaceuticals.

Experimental Animals

Swiss albino mice (17 to 23 g) of either sex were selected for acute oral toxicity and healthy young adult albino rabbits (1.5-2.5 kg) were used for the acute dermal irritation test. The animals were reared at animal house facilities of Pakistan Council of Scientific and industrial Research (PCSIR) Laboratories Complex, Karachi. The animals were housed individually for 14 days providing full access to the water as well as food following the examination in controlled environment. Food was removed 12 hours prior to experiment. Animals exhibiting abnormal/sluggish movements had been removed from research for any evidence of illness. The system and protocols for the usage of laboratory animals had been accepted by the ethical committee of PCSIR Laboratories Complex, Karachi (Notification no. ILD/TR-4582/2018 and ILD/TR-4583/2018).

Microorganisms

Tested organisms that used in the current study include 3 gram negative *Escherichia coli* (ATCC 012), *Klebsiella pneumonia* (ATCC 014), *Proteus vulgaris* (ATCC 074) and a gram Positive *Staphylococcus aureus* (ATCC6538). All the bacterial strains were purchased from the First Fungal Culture Bank of Pakistan, Institute of Agricultural Sciences (IAGS), Punjab University of Lahore except *S. aureus* that were taken from Microbiologics Inc. Laboratories.

Preparation of Inoculum

The 28 g of the nutrient agar were weighed and taken in the 1000 mL of distilled water, warmed continually until appearance of bubbles followed by sterilization at 121 °C as well as 15 psi in an autoclave for a period of 15 minutes. Bacterial

inoculums have been prepared on the nutrient agar from a 24 h pure culture. The 8 g of the nutrient broth were taken in 1 liter of the distilled water, dissolved and then sterilized in an autoclave at 121 ^{o}C as well as 15 psi for a period of 20 minutes. In a conical flask, 50 µL of culture stock solution was added in 50 mL of broth solution followed by the shaking over a horizontal shaker. Then spectroscopically the cell turbidity was measured after the duration of 24 h and compared to that of the 0.5 McFarland standards. After that, inoculum was ready in order to use for antibacterial activity.

Antibacterial Activity

To investigate the antibacterial potential of the plant extracts, disk diffusion method and well diffusion method were used.

Agar Disk Diffusion Assay

The agar disk diffusion strategy was utilized to investigate the antibacterial action of extracts as previously described[37] with minor modification. Succinctly, inoculum containing 10 CFU/mL was inoculated on Mueller-Hinton agar (MHA) petri plates for microbes. The sterile channel papers of 6 mm width having formulation Eczegone comprised of plant extracts of 50, 100 and 200 mg/mL in DMSO and DMSO as negative control were set on the outside of immunized agar plates with sterile forceps. Ciprofloxacin was utilized as reference anti-infection. The plates were placed into an incubator for 24 h at 37°C. The examples were tried in duplicates and the inhibitory zones were estimated in mili meter (mm) and average values were obtained.

Toxicity Study on Human Skin Keratinocytes HaCaT Cell Line

Cell culture and drug preparation. Human skin keratinocytes HaCaT cell line was procured from the Human Sciences Research Resources Bank (Japan Human Sciences Foundation, Tokyo, Japan). These cells were preserved in Dalbeccos Modified Eagles Medium (DMEM)-low glucose (Wako, Osaka, Japan) and 10% heat-inactivated fetal bovine serum (FBS) was added with 5% CO_2 humidified air at 37°C. After that, these cells were sub-cultured regularly for the experiments. Eczegone was dissolved in DMSO and then, finally it was utilized to make the desired indicated concentrations. The amount of DMSO in each samplewas kept less than 0.01%, and HaCaT cells were treated with increasing concentrations of Eczegone.

Morphological analysis. HaCaT cells were seeded in 6 well plate at density of 1.5 X 10^5 cells moreover treated with increasing concentrations of Eczegone (10 ng/mL-100 µg/mL) pursued by incubation for 24 hour at 37°C temperature. Afterward the cells were observed under microscope and pictures were taken to see if there is any morphological changes with the aforementioned drug.

Morphological cytotoxic assay. HaCaT cells were seeded in 6 well plate at density of 1.5 X 10^5 cells and treated with increasing concentrations of Eczegone (10 ng/mL-100 µg/mL) pursued by incubation for 24 h at 37°C. Afterward, the cells were washed twice with Phosphate Buffered Saline (-ve), fixed with 70% ethanol for 15 minutes and then stained with crystal violet dye (0.5%) for further 1 h. Stained cells were rinsed with tap water and dry at room temperature. The photo of the stained cells were taken by digital scanner.

Measurement of cell viability. HaCaT cells (4000 cells/well) were seeded in 96-well plate along withdealt with increasing concentrations of Eczegone (10 ng/mL-100 µg/mL) pursued by incubation for 24 h and 48 h at 37°C. Following the aforementioned time period, 20 µL of Methyl tetrazolium cytotoxicity (5 mg/ml) mixture was added into every well of the 96-well plates followed by the incubation at 37°C for 4 h. Then, 150 µL of dimethyl sulfoxide (DMSO) was added into everyone well, and in accordance withthe manufacturers guidelines, the absorbance was determined on a microplate reader at 490 nm. Survival ratio was calculated via normalizing the control groups.

Acute Oral Toxicity Experiment

Acute oral toxicity study of composite sample (*Azin, Spin, Fuin,* and *Lain* extracts) was determined in mice (OECD guideline No.423). Fasted animals (provided only water) for 12 h, were randomly partitioned into 6 groups having 6 animals in each group. GI, GII, GIII, GIV and GV served as test groups and received test drug at the different dosages of 5, 50, 300, 2000 and 5000 mg/kg body weight p.o by means of feeding cannula respectively, while at the same time GVI served as control group and received vehicle (distilled water) simply.

Observation. The animals were closely observed for any behavioral changes, toxicity signs and mortality, immediately after feeding of composite sample (*Azin, Fuin, Spin* and *Lain*) then after half hour, 4 h, 1 day, 2 day and then once a day for 14 days, all observation including skin changing, irritation of eyes and mucus membranes, convulsion tremors, sluggishness, diarrhea, salivations, sleep, coma as well as mortality rate was recorded.

Acute Dermal Irritant Test

To evaluate overall safety assessment of the product and potential of product or ingredients to cause skin erosion or irritation, the skin was often exposed either intentionally or unintentionally. Dermal irritation is defined as the production of reversible damage of the skin following the application of a test substance for up to 4 h (OECD TG404, 2002). It is generally assessed by the potential of a certain substance to cause erythema/scar and or oedema after a single topical application on rabbit skin and based on draize score (OECD TG404, 2002).

Preparation of animals. About 24 h before the start of experiment animals were placed and fur were removed by closely clipping the dorsal of trunk of the animals with care to avoid any abrading of skin.

Table 1. Standard Scoring System for Skin Reactions.

Reaction	Score
Erythema	
No erythema	0
Very slight erythema	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to scar formation	4
Oedema	
No oedema	0
Very slight oedema	1
Well defined oedema (edges of the area well defined by defined raising)	2
Moderate oedema (raising approximately 1 mm)	3
Severe oedema (raising more than 1 mm and extended beyond the area of exposure)	4
Total possible score for primary irritation	8

Table 2. Primary Dermal Irritaiton Index system.

Primary irritation index	Classification of irritancy
0 to 0.4	Non irritant
0.5 to 1.9	Slightly irritation
2 to 4.9	Moderate irritation
5 to 8	Severe Irritation

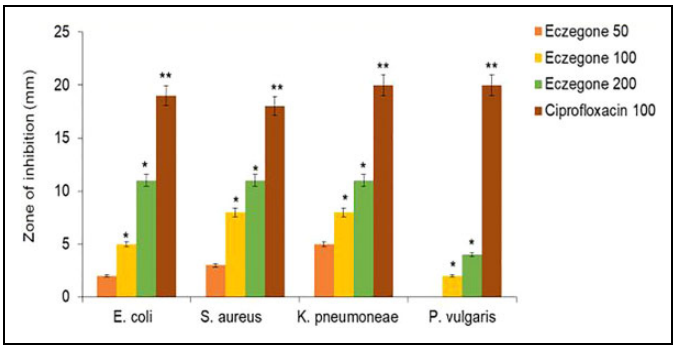

Figure 1. Antibacterial activity of Eczegone formulation by disk diffusion method.

Initial test. It was performed initially with 1 animal. The patch containing test substance weighing 0.5 g was applied over the test sides and held with non-adhesive dressings while untreated adjacent areas of skin was served as controlled. For initial test at 3 suitable sites the sample application was carried out and sample was removed at each of 3 time points; 3 min, 1 h and 4 h after application. At the end of exposure period the sample was removed by gentle swabbing with cotton wool soaked in distilled water. Skin reactions were observed timely at 1, 24, 48 and 72 h after removal of sample and responses were graded according to standard score system.

Confirmatory test. The test was repeated for an exposure period of 4 h using 3 additional animals to confirm the initial findings. The animals were examined at 1, 24, and 48 and 72 h for signs of erythema and oedema according to standard score system (Tables 1 and 2), after removal of patches animals were kept under the observation for 14 days to determine the reversibility of effects.

$$\text{Primary Dermal Irritation index (PDII)} = \frac{\text{Sum of erythema / oedema}}{\text{No. of test sites x grading interval}}$$

Statistical Analysis

Outcomes of the experiment were expressed as mean $\pm$ SD of duplicate or triplicate of the experiments. Statistical anasylysis was performed using 1-way ANOVA following bonferroni test and the p-value < 0.05 considered as significant.

Results

Antibacterial Activity

Antibacterial activity by disk diffusion method. Antibacterial activityof Eczegone formulation containing hydro-alcoholic extracts of *Azin, Spin, Fuin,* and *Lain* was experimented by utilizing disk diffusion method at various concentrations 50, 100, 200 mg/mL. Outcomes from the experiment revealed that the formulation containing all the plant extracts showed antibacterial effects at different concentrations. Highest result was shown against the *S. aureus, E. Coli, K.pneumoneae* and *P. vulgaris*, respectively at the dose of 200 mg/mL and is comparable with the control and standard. Our data revealed that all the bacterial strains were susceptible to the Eczegone formulation except *P. vulgaris.* (Figure 1 and Supplementary Figure 1A-D). Eczegone formulation showed lowest antibacterial activity against *P. vulgaris.* Hence, our data reported the antibacterial effect of the formulation Eczegone containing plants extracts, suggesting that this formulation can be used for the treatment of various skin ailments due to bacterial infections.

Effect of Eczegone on Human Skin Keratinocytes HaCaT Cell Line

Morphological analysis. In order to investigate the effect of eczegone on human skin keratinocytes HaCaT cell line, we had performed morphological analysis by treating the HaCaT cells with various indicated concentrations (10 ng/mL, 10 ng/mL, 1 µg/mL, 10 µg/mL and 100 µg/mL) of Eczegone and observed the morphology under microscope to see any morphological changes in the HaCaT cells after the 24 h of the incubation. Under microscope it was clearly seen that there was no changes in the morphology of the eczegone treated HaCaT cells. Hence, from the experiment it was found that samples were non-toxic against skin cells revealing that the drug has no cytotoxic effects on the morphology of skin keratinocytes (Figure 2).

Morphological cytotoxic assay. To further confirm the cytotoxicity of the eczegone, HaCaT cellswere treated with increasing

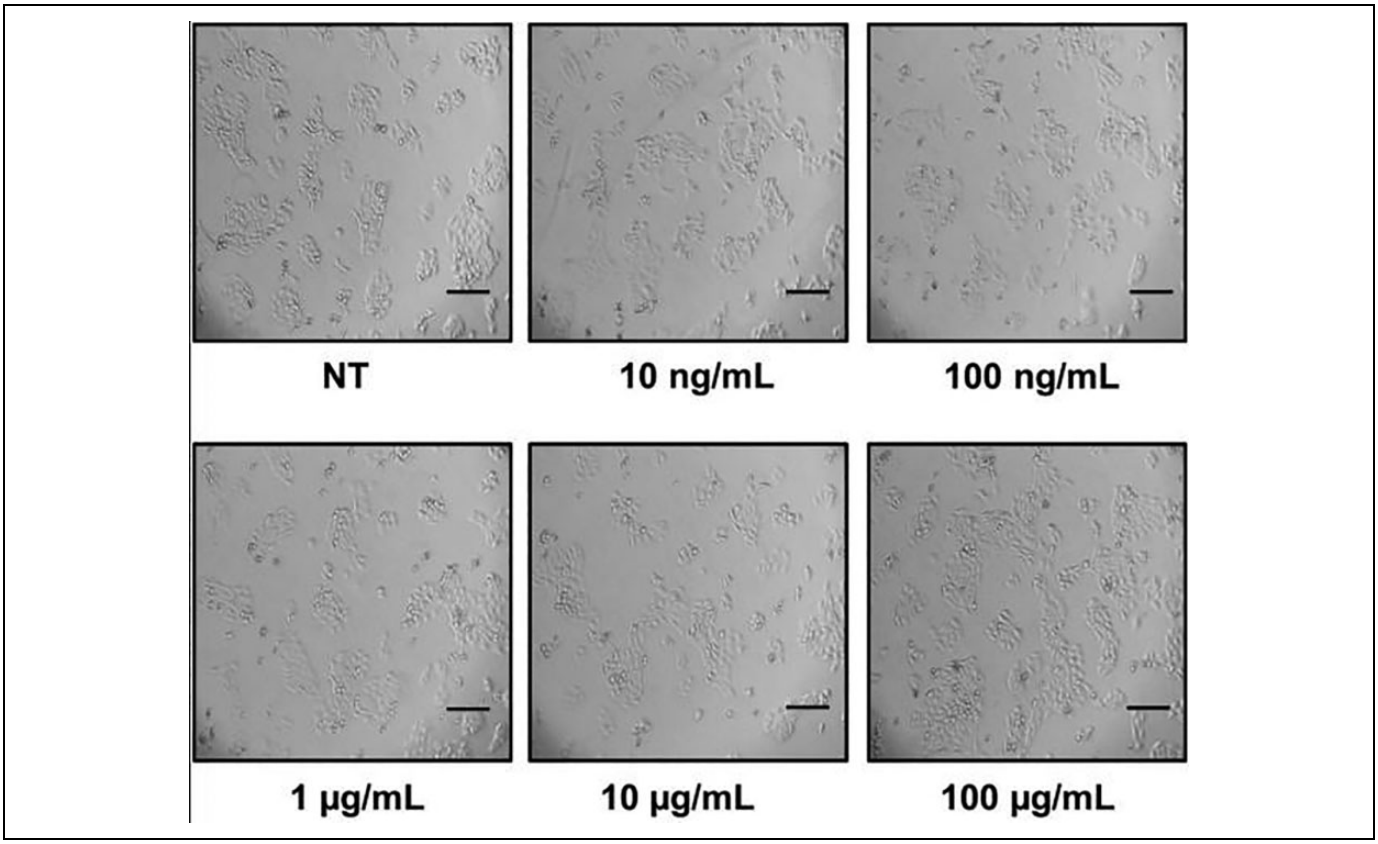

Figure 2. Morphological changes of HaCaT cells were examined under microscope after 24 h incubation with different indicated concentrations of Eczegone.100x magnification, scale bar; 100 µm. The experiment was repeated 3 times and these are the representative images.

concentrations of Eczegone (10 ng/mL-100 µg/mL) followed by incubation at 37°C for 24 h. Following the procedure the at the end the cells were stained with the crystal violet dye. Pictures taken through the digital scanner (Figure 3) from the experiment demonstrated that the crystal violet stained cells showed no sign of cytotoxicity on HaCaT cells. Hence, our data provided evidence that Eczegone has no cytototoxic effect on skins cell at various concentrations.

Measurement of the cell viability. Furthermore, to validate our results, we treated HaCaT cells with different indicated concentrations of Eczegone (10 ng/mL-100 µg/mL) for 24 h and 48 h, and then we determined cell viability using Methyl tetrazolium cytotoxicity (MTT) assay (Figure 4). The experiemental results revealed that Eczegone had almost no cell viability-reducing effects on the HaCaT cells, and therefore, it was considered to be safe for usage in further experimentation.Taken togather, the results from the experiments indicated the safety of the drug on skincells while using for the therapeutic purposes.

Effect of Eczegone on Acute Oral Toxicity

The acute oral toxicity study was assessed on the animal model by using different concentrations and observed for any kind of toxic/adverse effects. Eczegone was orally administered to the experimental animals with various dosages (5, 50, 300, 2000 and 5000 mg/kg body weight) for 2 weeks at different intervals. The results obtained from acute oral toxicity revealed that Eczegone did not show any toxicity signs such as irritation of skin, eyes, mucus membrane, behavior pattern, tremors, salivation, diarrhea and coma or mortality at maximum given dose level of 5000 mg/kg body weight at different intervals of the up to the 2 weeks (Table 3). Taken together, *in vitro* and *in vivo* study confirmed that Eczegone had no toxic effect and can be used for the management of Eczema.

Result of Acute Dermal Toxicity of Eczegone Ointment

To further evaluate the dermal toxicity of the Eczegone ointment, the ointment was tested on the healthy skin of the adult albino rabbits. The animals were observed initially for the interval of 1, 24, 48 and 72 h and then confirmatory study on additional animals for the period of 14 days. Observation from the experiment revealed that no signs of erythema and oedema were observed during the observation period of 14 days on the skin of the animals after topical application of the formulation as shown in Table 4. Hence, we concluded that Eastern Medicine coded medicinal formulation (Eczegone) is non-irritant, complies the standard method of acute dermal irritant test and seems to be safe for its topical application on the skin.

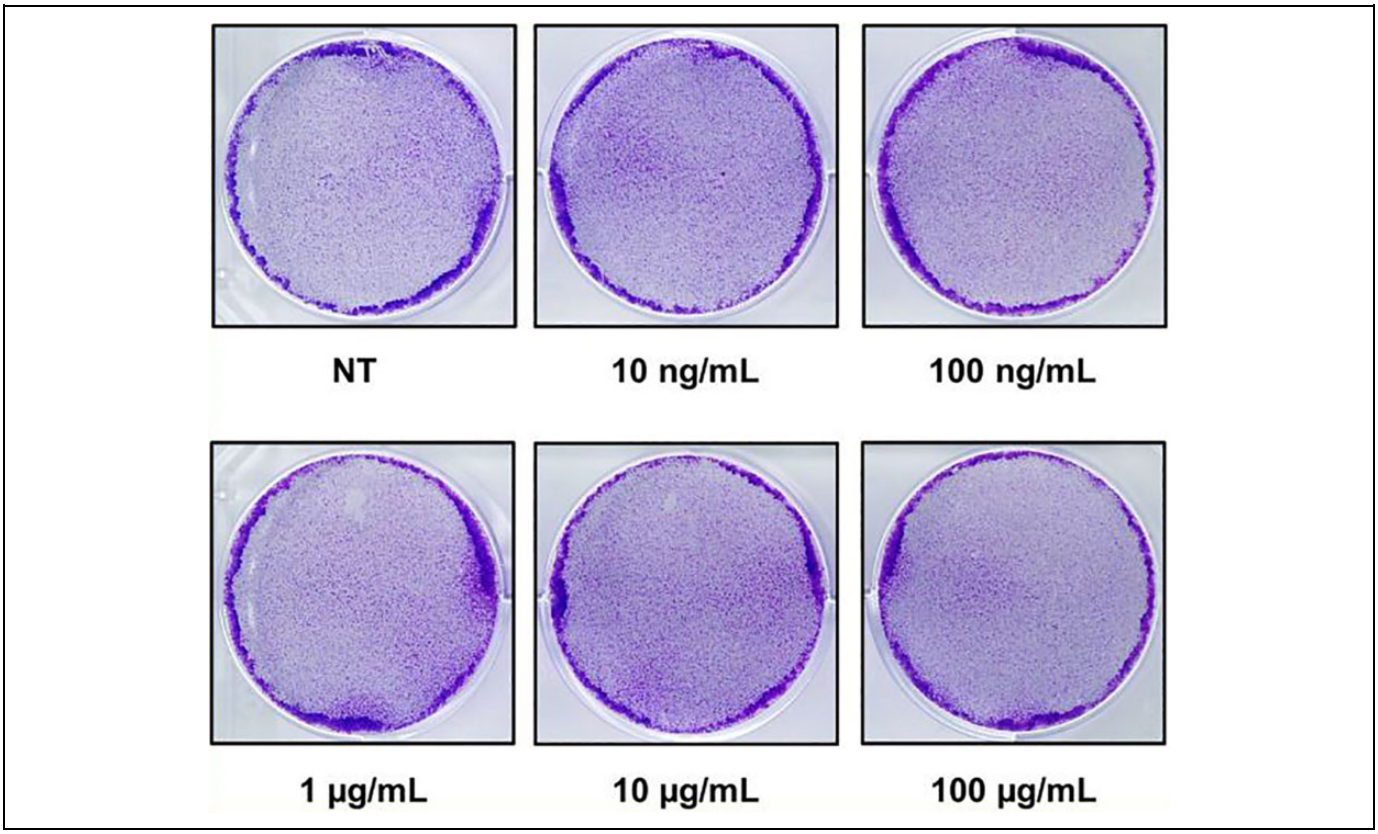

Figure 3. HaCaT cells were treated with the indicated concentrations of Eczegone for 24 h and stained with crystal violet dye to see the cytotoxic effects of the drug. The experiment was repeated 3 times and these are the representative images.

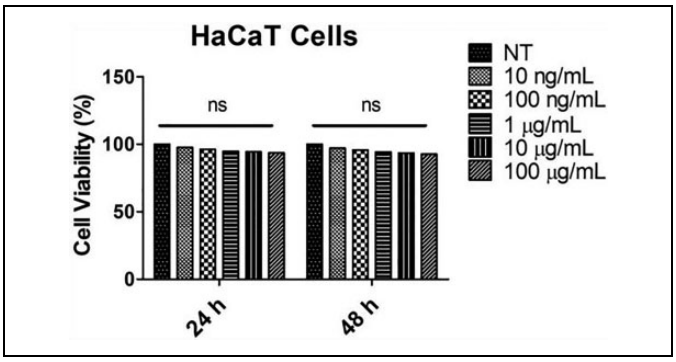

Figure 4. HaCaT cells were treated with the indicated concentrations of Eczegone for 24 h and 48 h, and cell viability was determined with the MTT assay. NT, Non-treated. The experiment was repeated 3 times and statistical anasylysis was performed using bonferroni test for group experiments. Here, ns indicated that $p > 0.05$ compared to NT.

Table 3. Observations of Acute Oral Toxicity of Composite Sample (*Azin, spin, Fuin* and *Lain*).

	30 mins		4 hrs		24 hrs		48 hrs		72 hrs	
Observations	C	T	C	T	C	T	C	T	C	T
Changes in skin and Fur	N	N	N	N	N	N	N	N	N	N
Changesin eyes	N	N	N	N	N	N	N	N	N	N
Changeson mucus Membrane	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N
Lethargy	N	N	N	N	N	N	N	N	N	N
Diarrhea	N	N	N	N	N	N	N	N	N	N
Tremors	N	N	N	N	N	N	N	N	N	N
Convulsion	N	N	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	N	N	N	N	N
Coma	N	N	N	N	N	N	N	N	N	N
Mortality	N	N	N	N	N	N	N	N	N	N

C: Control, **T**: Test, **N:** No .

Discussion

Eczema also referred as atopic eczema or atopic dermatitis is a chronic inflammatory dermal state clinically manifested by itchy red rash[38] along with eczematous lesions and pain of skin, sleep disturbances, various atopic and non-atopic co-morbidities.[39] However, in severe cases, it contains skin excoriation and secondary bacterial infections.[40] This clinical condition can be usually aggravated by different triggering factors like allergens, infections, seasonal and climatic variations or mental stress. Nearly 50% of the cases are usually identified in earlier first year of age but more than one third cases have a constant involvement in disease throughout their adulthood.[41,42] Eczema was estimated as of 2010 to affect about 230 million people

Table 4. Dermal Reactions (Time After Patch Removal).

	60 min(1 hr)				1st day (24 hr)				2nd day (48 hr)				3rd day (72 hr)				4th day			
	Ery		Oed		Ery		Oed		Ery		Oed		Ery		Oed		Ery		Oed	
Animal number	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	5th day				6th day				7th day				8th day				9th day			
	Ery		Oed		Ery		Oed		Ery		Oed		Ery		Oed		Ery		Oed	
	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	10th day				11st day				12nd day				13rd day				14th day			
	Ery		Oed		Ery		Oed		Ery		Oed		Ery		Oed		Ery		Oed	
	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

T: test, **C**: Control site, **Ery**: erythema, **Oed:** oedema

worldwide (3.5% of the population).[4] In the United States about 10% of children have eczema like condition, while in the United Kingdom about 20% are affected.[43]

Pruritus is the main physical complain in atopic dermatitis with complex etiology and can develop in any part of the body along with the oozing exudative eczematous lesions.[44] On the other hand, immune deregulation also remains an important factor in the development of atopic dermatitis, and the activation of type 2 immune responses is responsible for the impairment of the epidermal barrier.[45-50] In recent times, new pathophysiological approach of atopic dermatitis includes the abnormalities in lipid layer of epidermis, neuro-immune interactions, and microbial dysbiosis.[51,52] Atopic dermatitis has multifactorial etiology; so, the approach is to heal and protect the skin barrier mechanism and balancing the complex immuno-pathogenesis.[53]

Azin leaves have been used to make ointments, poultice and oil to treat different skin diseases like eczema, psoriasis, and scabies. Due to be blood purifier action its bark used to treat itching, acne and pruritus.[54] *Lain* has many therapeutic effects that were well documented these include, analgesic and anti-inflammatory action,[55] antibacterial,[56] antioxidant,[57] cytotoxic effects,[58] wounds and ulcers healing beside benefit in eczema and psoriasis[59] are known.

Sphaeranthus indicus (Gul-e-mundi) belongs to *Asteraceae* family. This plant can be used as a whole for medicinal purposes because all of its parts have medicinal value. In ayurveda or folk medicine, it is mainly used to treat different diseases. Plant powder is taken internally to treat skin diseases.[60] A paste is also made from this plant which is useful in treating itching and arthritis, filariasis (round worm caused infection), gout, edema and cervical adenopathy.[61] Juice is given orally for purification of blood and itching.[62] *Fain* is widely used in Ayurvedic system as well as unani system of medicine. In Ayurvedic system it is referred by the name of "Pitpapra" and in Unani system itis known by the name of "Shahtra." It is used as to purifier of blood in skin diseases and also has been used to applyexternally in leucoderma and swollen joints. The plant has been evaluated for the diverse pharmacological activities like anti-fungal,[63] antibacterial[64] as well as anti-inflammatory.[65] In Pakistan, this plant is used as traditionally for treatment of dermatological and topical diseases, cardio vascular and circulatory complaints, fever and headache.[66]

Upon the base of traditional use of the medicinal plants in the skin diseases, the Eastern Medicine coded medicinal formulation Eczegone comprising of *Azin, Fuin, Spin* and *Lain* hydro-alcoholic extracts were studied for antibacterial activity, acute toxicity, acute dermal irritant, crystal violet stained cells and MTT assays. Eczegone forulation showed antibactial activity against the tested bacterial strains and can be used in skin condition of bacterial origin. Eczegone considered to be safe and non-toxic invitro studites as it has shown no cytotoxic effects on human keratinocytes HaCaT Cells and non-toxic behavior in crystal violet stained cells and MTT assay.

Similarly upon its acute oral toxicity test, the Eczegone tablets has shown no side effects on skin, eyes and mucous membrane behavior pattern in Swiss albino mice at its maximal dose of 5000 mg/kg body weight while its ointment dosage form has also shown no signs of dermal reactions like erythema and oedema proving its non-irritant property upon local application in albino rabbits. Therefore, based on these findings Eastern coded medicinal formulation Eczegone can be applied for the prevention of eczema in further clinical studies.

Conclusion

Current study investigated antibacterial potential *in vitro* and *in vivo* toxicity, cell viability, and safety of the Eczegone formulation. The results of *in vitro* studies antibacterial, (HaCat cell line) crystal violet stained cells and MTT assay, the animal studies like acute oral and dermal toxicity tests in Swiss albino mice and albino rabbits have proven that Eastern coded formulation Eczegone tablet and ointment are safe, non-toxic and non-irritant. Eastern coded medicinal formulation Eczegone can be used for the prevention and treatment of eczema. Further studies are required to conduct its clinical clinical trails on large scale and to study its therapeutic efficacy in the treatment of eczema in future.

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Supplemental Material

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